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福建牡蛎(*Crassostrea angulata*)铜富集相关  
基因的研究

Studies on Genes Related to Copper Accumulation of the  
Fujian Oyster *Crassostrea angulata*

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## 摘要

牡蛎是具有重要经济价值的海水养殖品种，在世界很多地区都有养殖。而我国牡蛎养殖产量居世界首位。同时，牡蛎对铜、锌等金属具有很强的富集能力，经常被用于指示河口污染情况。随着工业发展，近岸河口区遭受越来越严重的金属污染，铜就是其中非常有代表性的金属污染物。因此牡蛎对铜的超富集现象越来越被人们所关注。学者们发现牡蛎对铜富集存在非常大的个体差异，然而相关的研究却非常有限，特别是缺少分子机制的解析。对这一现象的深入研究不但可以探寻、丰富牡蛎铜富集相关机制，更可以为选育牡蛎不同金属富集能力品系提供理论依据，有着非常重要的科研与生产价值。本研究选取我国南方牡蛎养殖的主要种类福建牡蛎(*Crassostrea angulata*)作为研究对象，对其铜富集个体差异进行分子生物学与实验生态学的研究，以转录组分析的方法寻找相关功能基因并进一步进行验证。并且进一步研究了福建牡蛎铜富集个体差异是否可以遗传给后代。本研究主要结果如下：

### 1. 福建牡蛎铜富集差异个体的转录分析

选取了两组存在铜富集差异(3-4倍)福建牡蛎的鳃和外套膜进行基因转录水平分析，共发现 372 个差异表达基因。对这些基因进行功能注释和 GO 富集，它们集中在神经递质载体蛋白基因、ATP 结合盒转运体(ABC)基因家族、多铜氧化酶基因以及一些脂类转运基因。而和铜毒性所引起的氧化损伤相关应激基因并没有差异表达。根据这些结果推测，牡蛎神经递质转运体基因的差异表达，可以影响牡蛎神经系统调控的生理行为，从而改变铜的富集动力学过程，产生铜富集差异现象。和铜离子结合、转运相关的基因表达量显著上调，则使铜富集量高的牡蛎个体能够保证细胞内铜稳态，免受铜的毒害。

### 2. 神经递质 GABA 转运体 2(GAT2)在牡蛎铜富集中的作用

根据转录分析结果,选取神经递质转运体 GAT2 基因作为潜在功能基因进行研究。首先克隆获得了 GAT2 基因 cDNA 片段。接着发现在该基因沉默后,牡蛎鳃和外套膜中铜富集量显著降低,而在 GABA 受体拮抗剂注射后牡蛎铜富集量显著升高,同时牡蛎滤水率和排氨率在 GABA 注射后呈现剂量依赖性降低。这表明,神经递质 GABA 可以调控牡蛎鳃和外套膜的生理行为,影响牡蛎对铜的富集,而神经系统中 GABA 浓度则由其转运体 GAT2 进行调控。因此,GAT2 基因的表达差异可能是引起牡蛎铜富集个体差异的关键因素。

### 3. 多药耐药基因 ABCB1 在维持牡蛎铜解毒中的作用

克隆获得福建牡蛎 ABCB1 基因 cDNA 全长 4544 bp,共编码 1370 个氨基酸。其结构中有典型的 ABC 家族保守结构域,与其他双壳类有较高的同源性。福建牡蛎 ABCB1 基因主要在鳃和外套膜的表皮柱状细胞中表达,在铜胁迫下,表达量迅速升高,呈现时间与铜暴露剂量依赖性表达模式。在铜暴露第 3 d 达到表达量高峰,最高浓度铜暴露组牡蛎鳃中表达量与对照组相比可达 5.61 倍( $P < 0.01$ )。低浓度铜暴露组中随着暴露时间的增长,该基因表达量逐步恢复到和对照相近水平,但是在高浓度暴露组,其表达量依然显著高于对照组。将 ABCB1 基因表达进行抑制后发现,牡蛎鳃和外套膜中铜的富集水平呈现显著增加。这些结果表明,牡蛎 ABCB1 可以将铜作为一种底物进行转运,推测其在牡蛎维持细胞铜稳态和铜解毒上发挥着重要作用。

### 4. 漆酶(Laccase)在牡蛎铜累积中的作用

对漆酶在多种生物基因组中的分布进行统计研究,发现牡蛎、扇贝的漆酶家族基因与人、昆虫、腹足类以及其他海洋生物相比,在基因组中有明显的扩张现象。克隆获得了福建牡蛎漆酶 1 类似基因(Lac1-like) cDNA 序列,全长 2760 bp,开放阅读框(ORF)编码蛋白含有 796 个氨基酸,其中有漆酶家族保守铜离子结合位点。该基因在鳃室顶侧表皮细胞与外套膜表皮柱状细胞中表达量较高。在不同浓度铜暴露实验中,Lac1-like 基因在鳃和外套膜中会被铜胁迫快速激动,表达显著上调,随着暴露时间延长,逐渐恢复到和对照组近似水平,呈现时间与铜

暴露剂量依赖性变化。在对该基因的表达进行干扰后，发现牡蛎鳃和外套膜中铜的含量显著降低，而镉的富集水平与对照相比没有显著变化。以上结果表明，Lac1-like 所结合的铜是牡蛎铜富集的重要组成部分，漆酶家族在牡蛎中的扩张与对铜的结合，也部分解释了牡蛎为何拥有如此强的铜富集能力。

## 5. 福建牡蛎铜富集能力遗传性

通过测定，选择铜富集有显著差异的牡蛎亲本组合产生家系。将后代家系在海上吊养 8 个月后进行外套膜金属含量以及整个软体部铜、锌、镉含量测定。发现家系间存在显著差异。而铜富集量高的亲本组合产生的后代，铜、锌富集量要高于低富集亲本组合所产生的后代，铜的富集平均值在外套膜中甚至高出 64.2%。同时，家系间以及个体间铜、锌的富集呈现显著正相关，相关系数高于 0.8，而镉则和它们没有显著相关性。这表明，牡蛎铜富集能力是可以遗传的，而且和锌富集水平有显著的正相关性。

**关键词：**福建牡蛎；铜富集；个体差异；基因表达；遗传

## Abstract

Oysters are one of the most important cultured mollusk species around the world. Based on the Fishery and Aquaculture Statistics 2012 (FAO Yearbook), Chinese oyster production accounted for 83% of the total production all over the world. Oysters are also considered as the hyper-accumulators of metals, especially for Copper (Cu), zinc (Zn). Metal pollution is a serious problem in the estuarine and coastal waters because of the development of economy, while Cu is one of vitally typical heavy metal pollutants. The variation of Cu among different individuals is ubiquitous, but the molecular mechanisms remain elusive. It is very useful to understand the mechanisms on metal accumulation in oysters, which will also provides the theoretical basis for the differences on metal enrichment abilities. In this study, the Fujian oyster *Crassostrea angulata* was selected as the research object because it is the most important cultivated oyster species and commonly distributed throughout coastal areas in south China. In this study, a comprehensive analysis of the transcriptome of *C. angulata* was conducted to reveal the relationship between gene expression and different Cu accumulation ability in oysters, then the potential functional genes were further studied. Otherwise, oyster families were established to study the inheritance of Cu accumulation ability. The main results are as follows:

### **1. Transcriptome analysis of *C. angulata* with differential Cu body burden**

The transcriptome difference among the oysters that displayed inter-individual variations (up to 4.3-fold) in Cu body burden was identified. A total of 139 differently expressed genes (DEGs) between the high-Cu and low-Cu concentrations in the gills were identified. In addition, 244 DEGs were detected in the mantle tissues. Gene ontology analysis for the differentially expressed genes showed that the

neurotransmitter transporter might affect the oyster behavior, which in turn led to differences in Cu accumulation. The oxidative stress related genes were detected in the eight DGE libraries, but were either not or differentially expressed in very small quantity. The ATP-binding cassette transporters superfamily (ABCs) played an important role in the maintenance of cell Cu homeostasis, vitellogenin, Laccase and apolipoprotein transport, and elimination of excess Cu.

## **2. The key role of GAT2 gene in the accumulation of copper in oyster**

According to the DEG analysis GABA transporter 2 (GAT2) gene was selected as potential functional gene. We obtained the partial *C. angulata* GAT2 cDNA segment. Gill and mantle Cu concentrations were significantly reduced after silencing the GAT2 gene, but significantly increased after the injection of GABA receptor antagonists. The oyster filtration rates and ammonia excretion rate were significantly reduced after GABA injection in a dose-dependent manner. It is revealed that GAT2 gene can regulate the inhibitory and excitatory actions elicited by GABA. The resulting behavioral changes may have an effect on Cu absorption and biodynamics, which led to individual variations in Cu accumulation.

## **3. Cu detoxification function of ABCB1 in *C. angulata***

The full-length transcript of ABCB1 gene was obtained by RACE from *C. angulata* gill and mantle tissues. The entire sequence of ABCB1 contains a 4113 bp open reading frame, encodes a putative protein of 1370 amino acids. It contained two ATP binding sites, which contained typical ABC transporter motifs. ABCB1 gene positive signals were detected in the apical compartment of cells lining the surface of the gills and in the epithelium of the mantle. A concentration-dependent highest *abcb1* mRNA level (up to 5.61-fold to the control) in gill and mantle existed across all Cu exposure concentrations after 3 days of Cu exposure. Then in the 30 µg/L Cu exposed group, ABCB1 expression level decreased to control levels at 15 d, but 100 and 300 µg/L Cu



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